CHARGE NUMBER: Project 1720

PROJECT TITLE: Physiochemical Morphology

PERIOD COVERED: October 1-31,1985

PROJECT LEADER: E. Thomas

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Objective: To determine the biochemical and biophysical properties of chloroplast submembrane preparations with respect to oxygen evolution and elucidate the degradation pathways of chloroplast proteins as a function of senescence. (V. Baliga and H. Nakatani)

Status: A procedure was developed to decrease the amount of LHCII component in triton-derived, PSII oxygen-evolving preparations. Thirty mM octyl-glucopyranoside lowered the levels of LHCII in PSII pellets derived from ultracentrifugation. Treatment with urea was also tested, and, while it removed the 33 kDa polypeptide, there was little effect on amount of LHCII. Electron transport and chlorophyll a/b ratios were measured on immature, ripe, and senescent leaves from Coker 319 tobacco. It was determined from these tests that both PSI and PSII reaction centers of the senescent leaf were still functioning, but that their antenna systems were degraded.

Plans: Studies will continue in the investigation of the differences in photosynthetic electron transport between green and senescent tobacco leaves. In another study, whole PSII particles will be separated from thylakoid membrane extracts using detergents other than triton. The oxygen evolving activity from purified components of PSII will be compared to the activity of intact photosynthesizing particles.

Objective: Study the physical and chemical properties of green tobacco and relate them to the mechanical properties of cured leaf. (E.Taylor, E. Thomas, J. Lyle, P. Echlin)

Status: Tobacco protoplasts at different stages of cell wall regeneration were studied by LM and SEM. In the LM studies Calcofluor, a cellulose-specific fluorescence stain, was used to visualize the relative degree of cell wall regeneration for a set of tobacco protoplasts. These protoplasts had been collected at different times during cell wall regeneration and preserved in 1% glutaraldehyde. Different preparation schemes were studied for their ability to preserve the cell wall features of tobacco protoplasts. These have included critical point drying and freeze-drying. Frozen-hydrated protoplasts were also prepared and examined by SEM. Individual features of the cell wall could not be distinguished because of the presence of surface ice.

Plans: Studies will continue on the imaging of the surface morphology of tobacco protoplasts using the SEM and TEM. Methods to remove surface water before freezing will be examined. In addition, freeze-dried samples prepared from frozen-hydrated protoplasts will be used with the higher-resolution STEM techniques.

SERVICE WORK: The particle size distribution of a sand sample was studied at the request of J. Lewis. The diameter of the sand, isolated from field tobacco, was determined using polarized light microscopy followed by video image analysis. In another request, image analysis techniques were used to determine the length histograms of tobacco shreds cut from different sizes of burley strip. This work was done in collaboration with the Engineering Department. At the request of T. Skidmore, MKS filler that was dried in either the ADT or the Hambro dryer was examined by SEM for differences in surface features. No differences were found.

- Eddie Harris